Determination of total Phosphorus, total Nitrogen and Nitrogen Fractions

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Acknowledgement

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Abbreviations

DIN: Deutsches Institut für Normung – German Institute of standardisation
d.w.: dry weight   f.w.: fresh weight
LOD: limit of detection   LOQ: limit of quantification
n.a. : not analyzed   MW: microwave   DS: desk study
Std.dev.: standard deviation   s rel.: relative standard deviation

INTRODUCTION

The present report describes evaluations of draft standards on the chemical parameters:
Total-Nitrogen, Kjeldahl-Nitrogen, Nitrogen Fractions: Ammonium and Nitrate and
Total Phosphorus in soil, biowaste and sewage sludge according to Desk study 16.

Total Nitrogen

The draft standard on determination of total nitrogen has to be evaluated for the three different matrices, here especially sewage sludges and treated biowaste. The small amount of test sample in Dumas combustion requires an intensive sample preparation and the demand on sample homogeneity has to be investigated. General method parameters (working range, detection limit, recovery, sample size, precision) will be described.

Kjeldahl Nitrogen

The draft standard on determination of Kjeldahl Nitrogen has to be evaluated for the three different matrices, especially for soil and biowaste. Investigations on the amount of sulfuric acid, amount and type of catalyst, heating time and heating temperature will be carried out for a semimicro-method. General method parameters (working range, detection limit, recovery, precision) will be described.

Nitrogen Fractions – Ammonium and Nitrate

The draft standard on determination of nitrogen fractions has to be evaluated for the three different matrices, here especially for soil and biowaste. Investigations on the type and amount of extraction solution (1M or 2M KCl), repetition of extraction, stability of test solutions and pretreatment of fresh and dry samples will be carried out. General method parameters (working range, detection limit, recovery, precision) will be described.

Total Phosphorus

The draft standard on determination of total phosphorus has to be evaluated for three different matrices. Two reflux methods (aqua regia and sulfuric acid/nitric acid) and two microwave assisted digestion methods (closed and open systems) will be evaluated. Shared actions on exchange of samples together with researchers working in the framework of desk study 19 (trace elements) are planned.

General method parameters (working range, detection limit, precision) will be described.

SUMMARY OF RESULTS

Total Nitrogen

The Dumas combustion method was evaluated for the analysis of total nitrogen in the concentration range 0 to 7.2 % N in soil, compost and sewage sludge. The LOQ (limit of quantification) for the determination of total nitrogen in real matrix samples was 0,02 % N, while the LOD (limit of detection) was 0,008 % N. Linearity was proven both by using a standard curve without matrix and by standard addition of nitrogen (in EDTA) to all matrices, seperately. Sample sizes of 250 mg soil and 100 mg compost and sewage sludge were used to obtain comparable detector response values. In general, relative standard deviations in precision experiments were lower than 10 %. Measurement uncertainty can be deduced from: weighing uncertainty, residual water in samples (2 – 12 %), impurities and fluctuations in the Dumas apparatus, e.g. in carrier gases.

Homogenisation of a soil sample (< 2 mm versus < 250 μm) did not cause significant differences in mean contents, though a small and significant difference in mean contents could be seen between a compost sample in < 2mm and < 250 μm quality. We therefore propose to use a quality of < 2 mm and to choose a milling quality of < 250 μm for all biowaste samples with high fibre content, as proposed earlier during the WEPAL validation (van Dijk, 2000) or to state other milling qualities in the documentation process, if necessary.

Kjeldahl Nitrogen

We describe a semimicro method for the determination of Kjeldahl nitrogen.
The ratio of sample to sulfuric acid of 1 : 10 allows good practical performance for all matrices and results in precision data with a standard deviation < 10 %.
Results for Kjeldahl nitrogen are generally lower than for Dumas nitrogen.
Nevertheless, results obtained from both methods differed by a maximum of 25 % for soil (SO13) and by a maximum of 10 % for biowaste / sludge.
Higher sample amounts in macromethods may be a good choice during routine work, especially as the use of 0,25 N compared to 0,01 N sulfuric acid in titration is preferable. Higher stability and reduced titration times are the advantages in the analysis of biowaste and sewage sludge samples. On the other hand measurement uncertainty is increased for samples with very low nitrogen contents using 0,25 N sulfuric acid in titration. Here, a selective use of 0,01 N for samples with a nitrogen content < 0,2 % is reasonable. Variations were tested for the amount of

catalyst added, the amount of sulfuric acid and heating time. The type of catalyst was evaluated for two multi component catalysts, containing K_2SO_4 and $CuSO_4$. The additional usage of selenium in catalyst mixtures did not result in higher yields compared to the additional usage of titaniumoxid. Furthermore selenium shows higher toxicity during laboratory handling.

While the amount of catalyst and heating time could be varied to large extent, the amount of sulfuric acid given for a micromethod in the draft standard (1,0g to 5 ml) was too low, resulting in insufficient dissolution of samples. Therefore we propose a minimum ratio of sample amount to sulfuric acid for the semicmicromethod of 1 to 10 and for the macromethod of 2 to 25.

Nitrogen Fractions

Extraction in 1M KCl solution provides higher stability of filtrates and higher recovery for ammonium compared to other extractants, such as water or 0,02M Calciumchlorid.

Analysis of KCl extracts can be done using both manual and automated methods based on spectrophotometric detection. Here we performed analysis using the CFA automated method.

Ion chromatographic detection is impossible due to interference of chloride from the extraction solution with the nitrate signale.

We tested variations in the ratio of sample to extractant, 1M versus 2M KCl extraction solutions, extraction time and storage of filtrates at two different temperatures (4°C and -18°C). The method chosen (1M KCl, ratio: 1: 10; extraction time: 1 x 1 hour; conventional filtration, storage at -18°C prior analysis and CFA analysis) showed good practical performance during routine work, high recovery values for nitrate (> 87%) and moderate recovery values for ammonium (> 75%) in all matrices tested.

Still, great variation in ammonium and nitrate contents: (0,2 mg/kg in soil and up to 700 mg/kg in sewage sludge) and contents of dry matter impose difficulties to both the extraction process and the analytical part.

Calibration and handling during laboratory work should be performed separately for soil, biowaste and sewage sludge in order to prevent cross contaminations

Repeated extractions improve recovery values, espacially for samples with high contents of ammonium/nitrate such as fresh biowaste and sewage sludges and for dried and ball milled soil samples used in validation.

Here, an alternative method using two extractions and filtrations or centrifugations is a method of choice for these sample catagories.

Total Phosphorus

We evaluated two different reflux digestion methods (aqua regia versus sulfuric acid/nitric acid) and two microwave digestion methods (aqua regia assisted heating in open and closed systems). No significant difference in contents of total phosphorus could be found for reflux versus microwave digestion or closed versus open microwave digestion.

Furthermore reflux digestion using sulfuric acid/nitric acid showed slightly increased but not statistically significant differences compared to reflux digestion in aqua regia.

We preferred conventional reflux digestion, as microwave assisted digestion using aqua regia causes corrosive damage to mechanical parts. Still, microwave heating may be a good choice for small sample amounts in small experimental series.

Variations in the ratio of sample amount to aqua regia was performed and as a result we propose a ratio of 1: 10 for soil samples and a ratio of at least 1 : 20 for biowaste and sludge samples for the analysis of phosphorus. In multielement analysis (phosphorus + trace elements) a ratio of 1 : 10 in reflux digestion is preferred, as concentrations of certain trace elements are near the LOQ.

Recovery was > 93% for all matrices tested and precision data showed a maximum relative standard deviation of 18 % for soil samples with low contents of total P and a maximum of 10 % for biowaste and sewage sludge samples.

CHAPTER 1

DETERMINATION OF TOTAL NITROGEN

1.   INTRODUCTION

The present report describes method evaluation and homogeneity tests for the determination of the chemical parameter: total nitrogen in three different matrices:

soil, biowaste/compost and sludge.

Determination was performed using the Dumas combustion method

For method details see Annex 2 in Desk Study 16

2.   MATERIALS AND METHODS

2.1   Apparatus:

Leco Instrumente GmbH, Kirchheim

Model FP428; Dumas Analyzer

2.2   Manufacturers equipment:

Tin crucibles for combustion , Leco
Ceramic crucibles, Leco
Lecosorb 20-30 mesh (NaOH on Silica, Leco)
Leco-Anhydrone ($MgClO_4$)
Copper – fillings, Leco
Combustion aid, Lactose, Leco
Quartz-wool, Leco

2.3 Chemicals:

Standard addition:

Ethylenedinitrilotetraacetic acid ; EDTA ($C_{10}H_{16}N_2O_8$) ;

(Titriplex II); Merck

MW = 292,25 g/mol ; purity : 99,4 – 100,6%

Calibration Standard :

"Nitrogen and sulfur in orchard leaves"; Leco

N % : 2,30 (+/- 0,04%) ; Lot No. : 492-110-2

Soil standard prepared and validated during ringtest validation 2003 (WEPAL)

83. Enquete FGII, VDLUFA

Standard 1: 0,031 % N (+/- 0,002 %N)

Standard 2: 0,31 % N (+/- 0,01 %N)

NIST standard; SRM 2781 (domestic sludge; 4,78 %N (+/- 0,11 %N)

2.4   Samples:

2.4.1 "Playground samples" from JRC, Ispra

Sample No.	Description	% N in SWCT report	Colour and Scent
SO 1	Brown soil, < 125 μm	0,25	light brown, sticky
SO 8	Mineralised soil, < 125 μm;	0,38	grey, sandy
SO 9	Sludge amended soil, < 125 μm	0,42	light brown, sandy
SO 13	Terra Rossa < 2 mm	0,39	light brown/grey
SO 13	Terra Rossa, additionally ball milled < 250 μm	0,39	light-brown/grey sticky
CW 1	Composted garbage, < 125 μm	1,50	dark brown,
CW 5	Compost, < 125 μm	1,52	dark brown,
SL 4	Sewage sludge, D < 125 μm	1,94	brown, strong scent low density
SL 11	Sewage sludge from electric Industry < 125 μm	0,65	light blue/green low density

Samples from ISPRA were dried at 105°C and ball milled; sample SO13 was ball milled in Kassel
% RW.: residual water determined by heating at 105°C over night:
RW (soils) < 2 % ; RW biowaste < 5 % ; RW sludge: SL 4 < 5%; SL11: 12 %

Kas 1*	Green leave compost ball milled < 250 μm fibrous, dark brown
Kas 2*	Biogas fermentation sludge ball-milled, < 250 μm fibrous, brown
Kas 3**	Green leave compost, ball milled / untreated < 250 μm / < 2 mm fibrous , brown

Characterisation and description of samples is given on page 89
Samples were dried at 105°C prior milling
    *   ringtest validation samples 2004, LUFA Kassel
    ** sample 2005 , LUFA Kassel

2.5   Sample pretreatment and homogeneity:

The influence of sample homogeneity and the milling/sieving process was investigated by precision measurement of the soil sample SO 13 (*Terra Rossa*) in both < 2 mm (ISPRA sample) and < 250 μm quality (ISPRA sample, milling and sieving was done in Kassel) and by precision measurement of compost sample Kas3 in both < 2 mm and < 250 μm quality.

In general, soil samples provided by JRC were powderized in one milling quality (< 125 μm) and did not contain visible foreign materials. Sewage sludge and biowaste samples contained higher amounts of organic matter and were of lower density than soil samples.

Samples from Kassel Kas1 and Kas2 (biowaste and fermentation sludge) were of milling quality of < 250 μm and included a higher visible fibre content. Preparation and pretreatment of samples from Kassel followed the guidelines given in van Dijk (2000).

Samples were air dried prior storage and sieved through a 2 mm sieve, dried at 105°C prior milling to 250 μm. Results are corrected for residual water contents, which is important for samples with low content of dry matter, especially sewage sludges.

2.6 Sample pretreatment and analysis (pilot studies):

Pilot studies done in Kassel during recent years showed, that pretreatment of forest soil samples at 40 °C and 105 °C did not change mean contents of total nitrogen significantly.

(Thieme, pers.commun ; data not shown). Furthermore pilot studies indicated, that the analysis of fresh soil samples is easily possible using the Dumas method and results are in good agreement with those obtained using dried samples. Nevertheless, usage of air dried samples allows best convenience during routine work and avoids losses of nitrogen due to prolonged heating at 105°C, which is a necessary procedure prior milling.

2.7 Analytical method evaluation:

Intrinsic method parameters (range, linearity, recovery) were determined for all matrices: soil, biowaste and sludge.The concentration range of the method was chosen up to a maximum content of 4,4 % N in sludge (in standard addition experiment) and up to a general maximum of 7.2% N (linearity of standard curve EDTA, diagrammes 1-4).

Recovery was estimated by standard addition of a synthetic nitrogen standard to samples of different matrices (soil, sewage sludge, biowaste).

The chemical standard used for standard addition was ethylenedinitrilotetraacetic acid ; ($C_{10}H_{16}N_2O_8$) ; (Titriplex II) ; MW = 292,25 g/mol ; purity : > 99,4 %; with a nitrogen content in EDTA of 9,59%.

Standard addition was performed by measurement of aproppriate amounts of EDTA together with 250 mg of soil sample or 100 mg of compost or sewage sludge sample from one combustion crucible. Three different maximum nitrogen contents were evaluated in the addition experiment, covering the expected range for each matrix: maximum: 2,2 % N in soil, 3,0 % N in compost; 4,4% N in sludge.

Different sample sizes were chosen in order to obtain similar detector responses both for samples with generally high (compost, sludge: 0,5% to 3,5% N) and low nitrogen contents (soil: 0.02% N to 0,5% N) The usage of combustion aids, e.g. lactose was tested by addition of lactose to a soil sample (SO8) during the evaluation process, and results did not differ from untreated samples (100mg SO8 +.50 mg Lactose: 0,404 %N +/- 0,05 %N; 100 mg SO8: 0,397 %N +/- 0,03 %N; three replicates) In general the use of combustion aids is recommended for samples with high nitrogen content (> 10 %) and low contents of organic matters, e.g. fertilizers.

The limit of quantification was determined by a calculation based on the standard deviation of a soil sample with very low nitrogen content (0,031 %) according to DIN 32645 and I.C.H. – guidelines (Table 5). The sample was measured in twelve subsamples and standard deviation was calculated. The LOQ could not be determined separately for compost and sludge in the same way, as no sample with similar low nitrogen content was available. Furthermore the Dumas method is based on weighing of subsamples, which excludes any dilution process to obtain low concentrations in the range of LOQ. Weighing of amounts smaller than 100 mg of sewage sludge and compost was not regarded equivalent to the above described method for LOQ determination.

Precision measurements were performed by the analysis of six subsamples of one sample on the same day. Day to day precision was investigated by additional measurement of four subsamples of one sample several days later.

Data analysis was done according to D.A.R. and I.C.H. guidelines (see Annex).

3.   RESULTS

3.1   Linearity

Linearity of the chemical standard EDTA in combustion was analyzed in the concentration range 0 – 7,2 mg N corresponding to a maximum content of 7,2 % N in sewage sludge and biowaste (calc. for 100 mg used in real matrix samples). Linearity was proven by a Pearsson coefficient of r = 0,99934. (x-axis: mg N in EDTA, determined by weight; y-axis: mg N in EDTA found in combustion ; Diagramme 1)

3.2   Limit of quantification

The LOQ was 0,02 % N, while the LOD was 0,008% N (see Table 5) .

3.3   Recovery

Recovery in soil varied between 100,0 % and 108,5 %.
Recovery in biowaste and sludge varied between: 94,5 % and 102,8 %.
(see Diagramme 2 – 4)

3.4   Precision

Soil, compost and sewage sludge samples of similar homogeneity and nitrogen contents between 0,2 % - 0,7% N (SO1, SO8, SO9, SL11) and 1,0 % - 2,2 % N (CW1, CW4, SL4, Kas1, Kas2) showed a standard deviation of 0,01 to 0,06 % N corresponding to a maximum relative standard deviation of 8 % (see Tables 1 – 4).

3.5   Milling quality

A milling and sieving test was performed by comparison of soil sample SO13 (*Terra Rossa*) in two different particle sizes (a) < 2 mm; (b) < 250 µm. Mean contents did not differ significantly. Compost sample Kas 3 was tested in two milling qualities: < 2 mm and < 250 µm.

The mean content of total nitrogen was slightly lower (1,78 +/-0,01 %N) and standard deviation was higher in the subsample with a particle size < 250 µm compared to the subsample with particle size < 2 mm.(1,86 +/- 0,07 %N) (Table 8).

3.6   Sample Size

The influence of sample size was evaluated by using both 100 mg and 250 mg subsamples of the ringtest validation standard soil: 0,31 % N. Sample size had a minor influence on mean contents of total nitrogen: 0,32% N found for sample size: 250 mg and 0,30% N found for sample size: 100 mg. Standard deviation was slightly increased in the subgroup: 100 mg (0,02% N versus 0,01% N) Nevertheless, a sample size of 250 mg for soil samples is appropriate, as nitrogen contents are lowest in soil compared to biowaste and sludge

(see Table 7).

Table 1:

Precision soil

SO1

Sample No.	% N
1	0,27
2	0,26
3	0,27
4	0,27
5	0,24
6	0,27

% N mean: 0,26 % N
Std deviation : 0,02 % N
S rel: 7,7%

SO8

Sample No.	% N
1	0,24
2	0,25
3	0,27
4	0,24
5	0,26
6	0,26

% N mean: 0,25 % N (see also Table 8.2, deviation from reported value in subsamples)
Std deviation: 0,01 % N
S rel: 4,0%

SO9

Sample No.	% N
1	0,46
2	0,47
3	0,47
4	0,46
5	0,46
6	0,44

% N mean: 0,46 % N
Std deviation: 0,01 % N
S rel : 2,2 %

Table 2:

Precision biowaste

CW1

Sample No.	% N
1	1,60
2	1,68
3	1,65
4	1,68
5	1,65
6	1,68

% N mean: 1,66 % N
Std deviation : 0,02 % N
S rel : 1,2 %

CW5

Sample No.	% N
1	1,70
2	1,69
3	1,70
4	1,71
5	1,72
6	1,64

% N mean: 1,69 % N
Std deviation: 0,03 % N
S rel: 1,8 %

Table 3:

Precision sewage sludge

SL4

Sample No.	% N
1	2,03
2	2,03
3	2,03
4	2,07
5	2,06
6	2,04

% N mean: 2,04 % N
Std deviation : 0,02 % N
S rel : 1,0 %

Continuation of Table 3

SL11

Sample No.	% N
1	0,74
2	0,70
3	0,72
4	0,75
5	0,72
6	0,73

% N mean: 0,73 % N
Std deviation: 0,02 % N
S rel: 2,7 %

Table 4:

Precision biowaste

Kas1

Sample No.	% N
1	1,22
2	1,25
3	1,27
4	1,28
5	1,19
6	1,26

% N mean: 1,24 % N
Std deviation : 0,04 % N
S rel: 3,2 %

Kas2

Sample No.	% N
1	2,19
2	2,13
3	2,20
4	2,22
5	2,07
6	2,19

% N mean: 2,17 % N
Std deviation: 0,06 % N
S rel : 2,8 %

Table 5 :

Estimation of LOQ

Analysis of soil standard: 0,031% N

Sample No during series	Std dev. % N
1	- 0,001
2	- 0,001
3	+ 0,001
10	- 0,005
11	+ 0,000
12	- 0,002
28	+ 0,002
29	- 0,003
59	+ 0,000
60	- 0,002
65	- 0,005
66	- 0,009

Mean standard deviation: 0,002 %N
S rel: 6,5 %

Table 6:

Precision of standard reference material

10.1) SL SRM 2781 sludge

Sample No.	% N
1	4,81
2	4,73
3	4,71
4	4,74
5	4,72
6	4,72

% N mean: 4,74 % N
Std deviation :   0,04 % N
S rel: 0,9 %

Table 7: Sample size variation

Sample size 250 mg

Soil standard 0,31% N / 03.12.2004

Sample No.	% N
1	0,33
2	0,32
3	0,32
4	0,33
5	0,30
6	0,32

% N mean: 0,32 % N
Std deviation: 0,01 % N
S rel: 3,1 %

Sample size 100 mg

Soil standard 0,31% N / 15.05.2005

Sample No.	% N
1	0,31
2	0,30
3	0,33
4	0,32
5	0,30
6	0,26

% N mean: 0,30 % N
Std deviation: 0,02 % N
S rel: 6,7 %

Table 8: Milling test

Soil SO13

Particle size: < 2 mm

Sample No.	% N
1	0,30
2	0,32
3	0,29
4	0,34
5	0,33
6	0,35

% N mean: 0,32 % N
Std deviation: 0,03% N
S rel: 9,4 %

Soil SO13 ;

Particle size: < 250 µm

Sample No.	% N
1	0,30
2	0,31
3	0,34
4	0,34
5	0,34
6	0,32

% N mean: 0,33 % N
Std deviation: 0,01 % N
S rel: 3,0 %

Compost Kas 3;

Particle size: < 2 mm

Sample No.	% N
1	1,85
2	1,96
3	1,86
4	1,87
5	1,90
6	1,74

% N mean: 1,86 % N
Std deviation: 0,07 % N
S rel : 3,8%

Compost Kas 3 ;

Particle size : < 250 μm

Sample No.	% N
1	1,79
2	1,80
3	1,75
4	1,78
5	1,78
6	1,75

% N mean: 1,78 % N
Std deviation: 0,01 % N
S rel: 0,6%

Table 9: Day to day precision

SO13 ;

Sample No during analysis	% N
mean subsample 1	*0,32*
1	0,30
2	0,30
mean subsample 2	*0,30*
3	0,30
4	0,31
mean subsample 3	*0,31*

% N mean: 0,31 % N
Std deviation: 0,01 % N
S rel: 3,2 %

SO8

Sample No during analysis	% N
mean subsample 1 (Table 1.2)	*0,25**
1	0,39
2	0,40
mean subsample 2	*0,40*

* see also Table 1.2 ; deviation from reported value in subsample 1 was checked by Kjeldahl analysis and could be confirmed.

CW1

Sample No during analysis	% N
mean subsample 1 (Table 2.1)	*1,60*
1	1,54
2	1,55
mean subsample 2	*1,55*
3	1,55
4	1,56
mean subsample 3	*1,56*

% N mean: 1,57 % N
Std deviation: 0,03 % N
S rel: 1,9 %

SL11

Sample No during analysis	% N
mean subsample 1 (Table 3.1)	*0,64*
1	0,67
2	0,66
mean subsample 2	*0,67*
3	0,66
4	0,66
mean subsample 3	*0,66*

% N mean: 0,66 % N
Std deviation: 0,02 % N
S rel: 3,0%

Diagramme 1 : Standard Curve EDTA
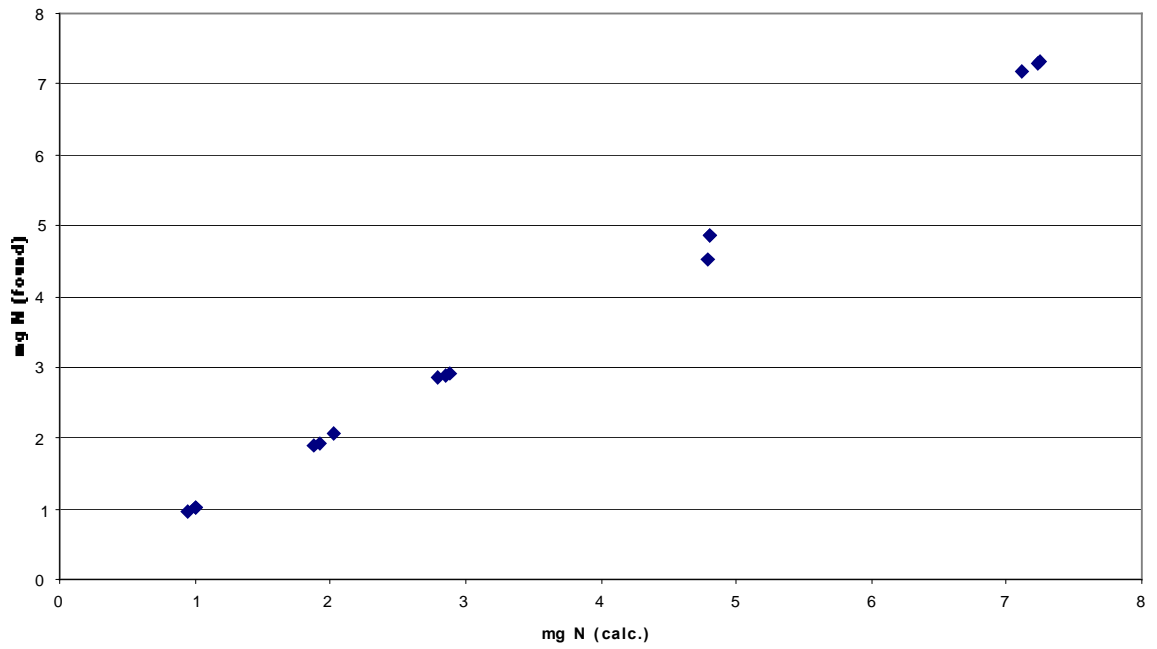
Curve parameters:
y intercept: 0,0003
slope of the curve: 1,004
Pearssons coefficient of regression: 0,99934

Diagramme 2 : Standard Addition Soil + EDTA
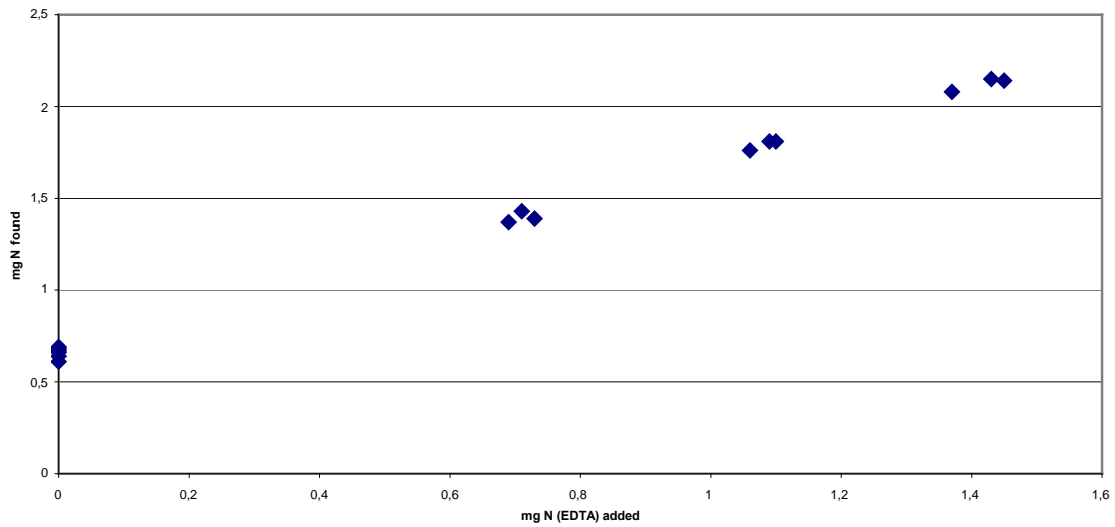
Curve parameters: y intercept: 0,66 ; slope of the curve: 0,962
Pearssons coefficient of regression: 0,99931

Diagramme 3 : Standard Addition Biowaste + EDTA
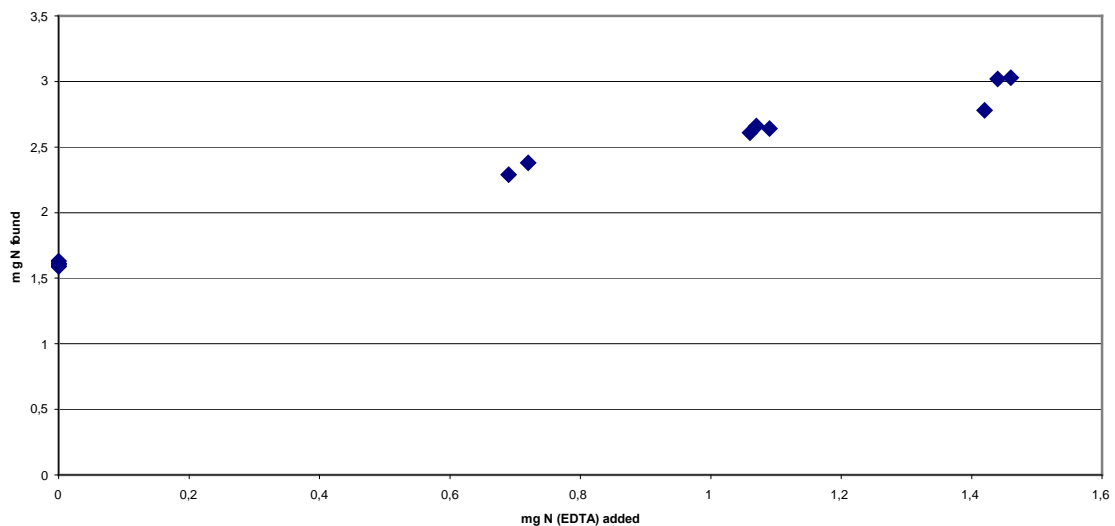
Curve parameters: y intercept: 1,62 ; slope of the curve: 1,04
Pearssons coefficient of regression: 0,99496

Diagramme 4 : Standard Addition Sludge + EDTA
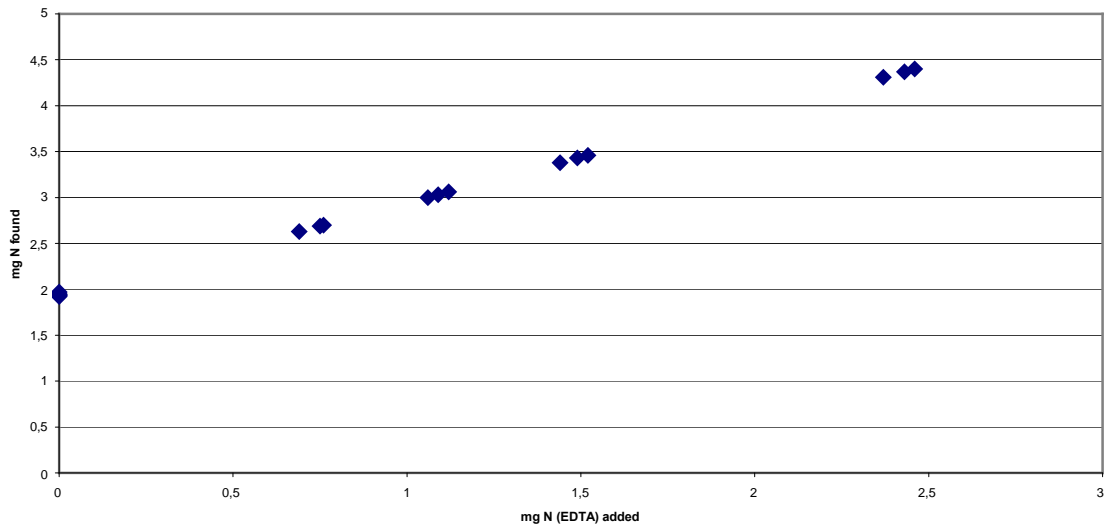
Curve parameters: y intercept: 1,94 ; slope of the curve: 0,99926
Pearssons coefficient of regression: 0,99994

4.   SUMMARY

The Dumas combustion method was evaluated for the analysis of total nitrogen in the concentration range 0 to 7.2 % N in soil compost and sewage sludge. The LOQ (limit of quantification) for the determination of total nitrogen in real matrix samples was 0,02 % N, while the LOD (limit of detection) was 0,008 % N. Linearity was proven both by using a standard curve without matrix and by standard addition of nitrogen (in EDTA) to all matrices, seperately. Sample sizes of 250 mg soil and 100 mg compost and sewage sludge were used to obtain comparable detector response values. In general, relative standard deviations in precision experiments were lower than 10 %. Measurement uncertainty can be deduced from: weighing uncertainty, residual water in samples (2 – 12 %), impurities and fluctuations in the Dumas apparatus, e.g. in carrier gases.

Homogenisation of a soil sample (< 2 mm versus < 250 μm) did not cause significant differences in mean contents, though a small and significant difference in mean contents could be seen between a compost sample in < 2mm and < 250 μm quality. We therefore propose to use a quality of < 2 mm and to choose a milling quality of < 250 μm for all biowaste samples with high fiber content, as proposed earlier during the WEPAL validation (van Dijk, 2000) or to state other milling qualities in the documentation process, if necessary.

CHAPTER 2

DETERMINATION OF KJELDAHL - NITROGEN

1   INTRODUCTION

The present report describes scientific investigations and method evaluation for the determination of the chemical parameter: Kjeldahl - nitrogen in three different matrices: soil, biowaste/compost and sewage sludge.

For method details see Annex 4 in Desk Study 16

2   MATERIALS

2.1   Apparatus:

Gerhardt Instrumente GmbH, D-Königswinter
Digestion System and Destillation system: Vapodest

2.2   Equipment:

Digestion Tubes of nominal volume 280 ml
Nitrogen free paper crucibles

2.3   Chemicals:

All Chemicals were purchased from Merck – VWR International

Standard addition:

Glycine; ($NH_2CH_2CO_2H$) ; MW = 75,07 g/mol

purity : > 99,7 %

Soil standard prepared and total N validated by Dumas combustion analysis during ringtest validation 2003 (WEPAL)

83. Enquete FGII, VDLUFA

Standard 1: 0,031 % N (+/- 0,002 %N)

Standard 2: 0,310 % N (+/- 0,010 %N)

H_2SO_4 ; 98 % ; ρ = 1,84 kg/l

H_2SO_4 ; 0,25 N = 0,125 M

H_3BO_3 p.a. ;

NaOH ;   32 %

$(NH_4)SO_4$ p.a.

TiO_2 p.a. (granules 1-4 mm)

K_2SO_4 p.a.

$CuSO_4$ p.a.

Se (black) p.a. > 99%

2.4   Preparation of reagents:

Catalyst mixtures: (were grinded in a mortar each day before use)
Mix 1: K_2SO_4 : $CuSO_4$ - 10 : 1
Mix 2: K_2SO_4 : $CuSO_4$: TiO_2 - 10 : 1 :1
Mix 3: K_2SO_4 : $CuSO_4$: Se - 10 : 1 : 1

H_3BO_3 solution for titration: 20 g/l + 5g/l K_2SO_4 (heated gently for dissolution)
$(NH_4)SO_4$ for calibration: 20 g / l + 4 ml H_2SO_4 conc/l

2.5   Samples:

Playground samples » from JRC, Ispra

Sample No.	Description	% N in SWCT report	Colour and Scent
SO 1	Brown soil, < 125 μm	0,25	light brown, sticky
SO 8	Mineralised soil, < 125 μm	0,38	grey, sandy
SO 9	Sludge amended soil, < 125 μm	0,42	light brown, sandy
SO 13	Terra Rossa < 2 mm	0,39	light brown/grey
CW 1	Composted garbage, < 125 μm	1,50	dark brown,
CW 5	Compost < 125 μm	1,52	dark brown,
SL 4	Sewage sludge, D < 125 μm	1,94	brown, strong scent low density
SL 11	Sewage sludge from electric Industry < 125 μm	0,65	light blue/green low density

Samples from ISPRA were dried at 105°C and ball milled, % N was determined by combustion analysis – and does not give Kjeldahl % N
% RW.: residual water determined by heating at 105°C over night:
RW (soils) < 2 %; RW biowaste < 5 % ; RW sludge: SL 4 < 5%; SL11: 12 %

Continuation Table 2.5

Samples from Kassel

Kas 1*	Green leave compost ball milled < 0,25 mm fibrous, dark brown 1,24 % N (+/- 0,04)
Kas 2*	Biogas fermentation sludge Ball-milled, < 0,25 mm fibrous, brown 2,17 % N (+/- 0,06)
Kas 3**	Green leave compost, Ball milled < 0,25 mm fibrous , brown 1,78 % N (+/-0,01)

Characterisation and description of samples are given on page 89
Samples were dried at 105°C prior milling; % N was determined by Dumas combustion
*   ringtest validation samples 2004 (Schaaf, 2004)** sample 2005 (LUFA Kassel, test sample)

2.6   Sample pretreatment:

In general, soil samples provided by JRC were powderized in one milling quality (< 125 μm) and did not contain visible foreign materials. Sewage sludge and biowaste samples contained greater amounts of organic matter and were of lower density than soil samples.

Samples from Kassel: Kas1 and Kas2 (biowaste and fermentation sludge) were of sieve quality of < 250 μm and included a higher visible fibre content. Preparation and pretreatment of samples from Kassel followed the guidelines given in van Dijk (2000).

Samples were air dried prior storage and sieved through a 2 mm sieve, dried at 105°C prior milling to 250 μm. Results are corrected for residual water contents, which is important for samples with low content of dry matter, espacially sewage sludges.

2.7   Analysis:

An amount of 1,0000 (+/-0,0001) g of sample was weighed into paper crucibles free of nitrogen and filled into digestion tubes together with the paper crucible. 2,5 g of solid catalyst and finally 10 ml of sulfuric acid were added.

On the day of analysis 20 flasks were prepared and digestion was done using the appropriate temperature programme over night. Addition of catalyst results in temperature increase, so that temperatures given in the following tables are programmed temperatures and not reaction temperatures. In general, the digestion must not be performed at temperatures > 400 °C

Reaction mixtures using sample amounts greater than 1 g were placed at room temperature for at least 24 hours in order to keep the digestion process under control. The next day, distillation was performed after calibration of the distillation procedure with ammoniumsulfat. Two blank tests using tubes without addition of chemicals were carried out before calibration and analysis. Distillation lasted 3 min 30 sec per sample and the end point of distillation was measured potentiometrically.

Digestion programme used for evaluation:

No 1:

Temperature / °C	Duration / hours
200	1
300	0,5
400	1,2

Digestion programme used for sample amounts greater than 1,0 g :

No 2:

Temperature / °C	Duration / hours
150	1
200	1
250	0,3
300	0,3
400	1,2

2.8   Analytical method evaluation:

Intrinsic method parameters (range, linearity, recovery) were determined for all matrices: soil, biowaste and sludge.The concentration range of the method was chosen up to a maximum content of 6,0 % N in standard addition experiments to real matrix samples.

The chemical standard used for standard addition was the amino acid glycine; purity : 99,7 % with a nitrogen content in glycine of 18,7 %.

Standard addition was performed by measurement of aproppriate amounts of glycine (max 240 mg) together with 1,0 g of sample into one paper crucible

The limit of quantification was determined by a calculation based on the standard deviation of a soil sample with very low nitrogen content (0,031 % N) according to DIN 32645 and ICH –guidelines.The sample was measured in ten subsamples and standard deviation was calculated. The LOQ could not be determined separately for compost and sludge in the same way, as no sample with similar low nitrogen content was available. Weighing of amounts smaller than 1 g of sewage sludge and biowaste was not regarded equivalent to the above described method for LOQ determination, as this results in an overall variation of the digestion process..

Precision measurements were performed by the analysis of three subsamples on two days.

Data analysis was done according to D.A.R. and I.C.H. guidelines (see Annex).

3   RESULTS:

3.1   Scientific investigations:

Amount of sulfuric acid:

A ratio of 1 : 10   (w/V) was chosen, as lower amounts of sulfuric acid (1: 7,5) resulted in insufficient dissolution of solid materials. A ratio of of 1: 10 yielded a clear supernatant, while a ratio of 1 : 7,5 yielded a brown mass without supernatant. A clear supernatant indicates complete digestion.

Amount of catalyst:

The amount of catalyst added was chosen in a ratio of sample to catalyst: 1 : 2,5.

The use of varying amounts of sample to catalyst was evaluated in the range: 1 : 0,25 to 1 : 2,5

Reduction of the total amount of catalyst from 2,5 g to 0,25 g resulted in a maximum decrease of nitrogen contents of 10 %. (Table 6)

Type of catalyst:

Two catalyst mixtures were evaluated and as a result we propose the usage of a mixture of $K_2SO_4/CuSO_4/TiO_2$ (Table 8). We used a grinded catalyst mixture in the ratio: 200: 20: 20, while the reported mixture is in the ratio 200: 6: 6. Nevertheless, we were able to lower the total amount of catalyst to 2,5 g per analysis.

Substitution of titaniumoxid by selenium did not change contents of Kjeldahl nitrogen in soil and sewage sludge samples. The biowaste sample chosen (CW5) showed a significantly higher content of nitrogen when digested with selenium compared to titaniumdioxid catalyst (t-Test, 99% confidence). Nevertheless, differences were very small: 1,52 % N (+/- 0,02) compared to 1,59 % N (+/- 0,02) and test results would have been statistically insignificant when using measurement uncertainties given by recovery values of 102 % in calculation of mean values. Furthermore the use of selenium should be avoided due to its high toxicity.

(Table 7)

Heating programme
The heating programme No 1. showed good practical performance for low sample amounts.
Sample amounts greater than 1,0 g should be prehandled as described above and heating should
be performed using programme No. 2.

3.2 Analytical method evaluation:

Linearity
Linearity of the chemical standard glycine was analyzed in the concentration range:
0 – 200 mg N corresponding to a maximum content of 3,7 % N in sludge and biowaste
(calc. for 1,0 g used in real matrix samples). Linearity was proven by a Pearsson coefficient of
r = 0,9997. (x-axis: mg N in glycin determined by weight; y-axis: mg N in glycine, see
Diagramme 1)In standard addition experiments a maximum range of 6 % was evaluated and
linearity was proven by Pearsson coefficients of r > 0,999.

Limit of quantification

The LOD was 0,03 % N, while the LOQ was 0,1 % N (see Table 5)

Recovery

Recovery in soil, sludge and biowaste varied between 93,6 % N and 102,6 % N.
(Table 9)

Precision

Soil , compost and sludge samples of similar homogeneity and nitrogen contents between
0,2 % - 0,7% N (SO1, SO8, SO9, SO13, SL11) and 1,0 % - 2,2 % N (CW1, CW4, SL4, Kas1,
Kas2) showed a standard deviation of 0,01 % N to 0,03 % N corresponding to a maximum
relative standard deviation of 10 % (see Tables 1 – 4) .

Table 1: Analysis of soil samples / ISPRA

SO1

Sample No.	% N
1	0,24
2	0,24
3	0,23
4	0,24
5	0,24
6	0,24

% N mean: 0,24 % N
Std deviation : 0,01 % N
s rel: 4,2 %

SO8

Sample No.	% N
1	0,23
2	0,23
3	0,21
4	0,24
5	0,22
6	0,20

% N mean: 0,22 % N (deviation from reported value - ISPRA)
Std deviation: 0,02 % N
s rel.: 9,1 %

SO9

Sample No.	% N
1	0,41
2	0,40
3	0.40
4	0,39
5	0,39
6	0,39

% N mean: 0,40 % N
Std deviation: 0,01 % N
s rel: 2,5 %

SO13

(< 2 mm)

Sample No.	% N
1	0,27
2	0,27
3	0,29
4	0,32 * outlier
5	0,28
6	0,28

* outlier
% N mean: 0,28 % N
Std deviation: 0,01 % N
s rel.: 3,6 %

Table 2: Precision biowaste

CW1

Sample No.	% N
1	1,44
2	1,47
3	1,42 *
4	1,48
5	1,48
6	1,51

* outlier
% N mean: 1,48 % N
Std deviation : 0,03 % N
s rel : 2,0 %

CW5

Sample No.	% N
1	1,53
2	1,56
3	1,54
4	1,49
5	1,52
6	1,50

% N mean: 1,52 % N
Std deviation: 0,03 % N
s rel: 2,0 %

Table 3: Precision sewage sludge

SL4

Sample No.	% N
1	1,92
2	1,92
3	1,93
4	1,91
5	1,92
6	1,95

% N mean: 1,92 % N
Std deviation : 0,02 % N
s rel: 1,0 %

SL11

Sample No.	% N
1	0,68
2	0,68
3	0,62
4	0,62
5	0,68
6	0,70

% N mean: 0,66 % N
Std deviation: 0,03 % N
s rel: 4,5 %

Table 4:Precision of additional compost and sludge samples / Kassel

Kas1

Sample No.	% N
1	1,15
2	1,10
3	1,11
4	1,17
5	1,14
6	n.a.

% N mean: 1,13 % N
Std deviation : 0,03 % N
s rel: 2,7 %

Continuation of Table 4:

Kas2

Sample No.	% N
1	2.02
2	2,00
3	2,03
4	2,03
5	2,00
6	n.a.

% N mean: 2,02 % N
Std deviation: 0,02 % N
s rel: 1,0 %

Table 5:
Estimation of LOQ
Analysis of soil standard: 0,031 % N

Sample No during series	Std dev. % N
1	+ 0,025 *
2	- 0,000
3	+ 0,014
4	- 0,000
5	- 0,000
6	- 0,000
7	- 0,014
8	- 0,014
9	- 0,014
10	- 0,000

Mean standard deviation: 0,01   % N
* outlier

Table 6: Variation: Catalyst amount

Sample: CW 5

Catalyst amount (g)	% N / Colour *
2,50	1,52 (+/- 0,04) / light blue
1,00	1,45 (+/- 0,01) / light brown
0,50	1,41 (+/- 0,02) / brown
0,25	1,38 (+/- 0,01) / dark-brown

Each data point represents the mean of three digestions.
Sample amount : 1,0 g
* Colour is given for the residue after addition of NaOH and destillation, blue colour indicates complete digestion

Table 7 :Variation: Catalyst Type

K_2SO_4 / $CuSO_4$ / TiO_2 (10/1/1) *	K_2SO_4 / $CuSO_4$ / Se (10/1/1) **
SO1 = 0,24 (+/- 0,01) %N	SO1 = 0,25 (+/- 0,01) %N
CW5 = 1,52 (+/- 0,02) %N	CW5 = 1,59 (+/- 0,02) %N
SL11 = 0,60 (+/- 0,01) %N	SL11 = 0,61 (+/- 0,01) %N

* Each data point represents six analysis (see precision)
* * Each data point represents three analysis

Table 8: Variation: Heating programme

Programme 1 *	Programme 2 **
SO1 = 0,24 (+/- 0,01) %N	SO1 = 0,22 (+/- 0,01) %N
CW1 = 1,40 (+/- 0,04) %N	CW1 = 1,36 (+/- 0,02) %N
SL11 = 0,60 (+/- 0,01) %N	SL11 = 0,59 (+/- 0,01) %N

* Each data point represents six analysis (see precision)

* * Each data point represents three analysis

Diagramme 1 : Standard Curve Glycine
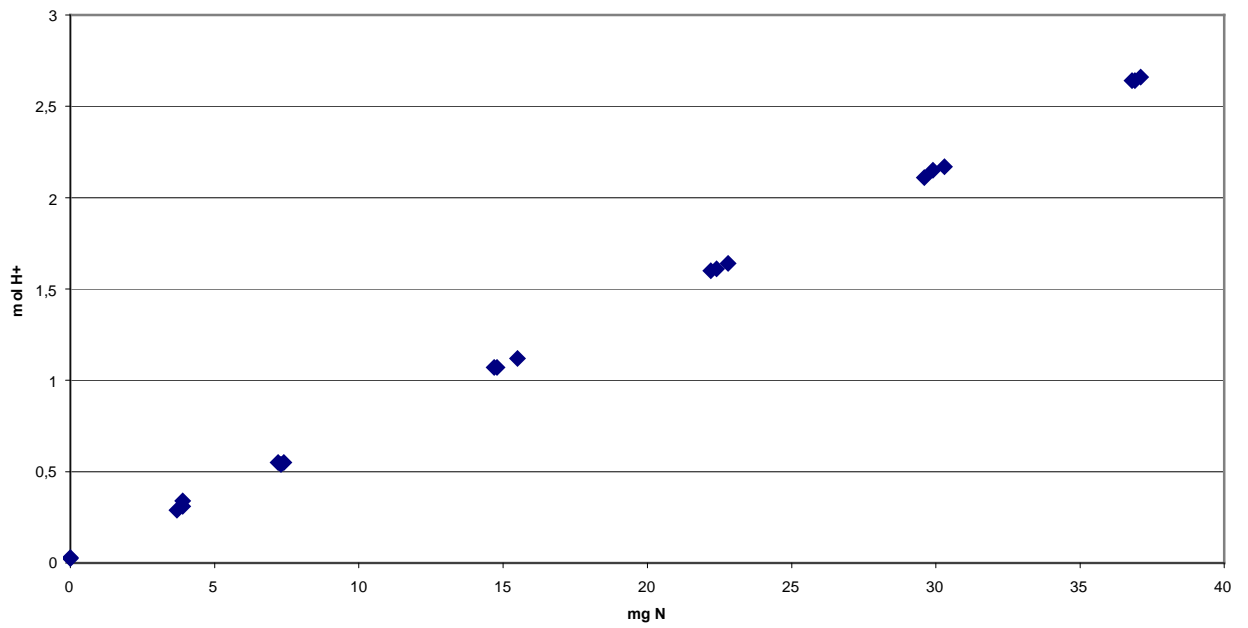
Curve parameters:
y intercept: 0,025
slope of the curve: 0,0705
Pearssons coefficient of regression: 0,9997

Table 9: Recovery after standard addition of sample matrix with glycine

Sample	Recovery % **
SO1 + 1,5 % N	96,4 (+/- 3,5)
CW1 + 1,5 % N	101,4 (+/- 0,7)
CW1 + 2,7 % N	101,2 (+/- 1,4)
CW1 + 4,7 % N	98,6 (+/- 3,9)
SL11 + 1,5 % N	99,6 (+/- 1,0)
SL11 + 2,7 % N	99,8 (+/- 0,2)
SL11 + 4,7 % N	99,6 (+/- 0,2)

** each data point represents three analysis

Standard addition Soil SO1 + Glycine

Curve parameters:
y intercept: 0,25
slope of the curve: 1,012
Pearssons coefficient of regression: 0,9997

Standard Addition Compost CW1 + Glycine

Curve parameters:
y intercept: 1,37
slope of the curve: 0,9977
Pearssons coefficient of regression: 0,99991

Standard Addition Sewage Sludge + Glycine

Curve parameters:
y intercept: 0,61
slope of the curve: 1,005
Pearssons coefficient of regression: 0,99996

3   SUMMARY

The present report describes a semimicro method for the determination of Kjeldahl Nitrogen.

The ratio of sample to sulfuric acid of 1 : 10 allows good practical performance for all matrices and results in precision data with a standard deviation < 10 %.

Results for Kjeldahl nitrogen are generally lower than for Dumas Nitrogen.

Nevertheless, results obtained from both methods differed by a maximum of 25 % for soil (SO13) and by a maximum of 10 % for biowaste / sludge.

Higher sample amounts in macromethods may be a good choice during routine work, especially as the use of 0,25 N compared to 0,01 N sulfuric acid in titration is preferable. Higher stability and reduced titration times are the advantages in the analysis of biowaste and sewage sludge samples. On the other hand measurement uncertainty is increased for samples with very low nitrogen contents using 0,25 N sulfuric acid in titration. Here, a selective use of 0,01 N for samples with a nitrogen content < 0,2 % is reasonable. The amount of catalyst added, the amount of sulfuric acid and heating time was varied. The type of catalyst was evaluated for two multi component catalysts, containing K_2SO_4 and $CuSO_4$. The additional usage of selenium in catalyst mixtures did not result in higher yields compared to the additional usage of titaniumoxid. Furthermore selenium shows higher toxicity during laboratory handling.

While the amount of catalyst and heating time could be varied to large extent, the amount of sulfuric acid given for a micromethod in the draft standard (1,0g to 5 ml) was too low, resulting in insufficient dissolution of samples. Therefore we propose a minimum ratio of sample amount to sulfuric acid for the semicmicromethod of 1 to 10 and for the macromethod of 2 to 25.

CHAPTER 3

DETERMINATION OF NITROGEN FRACTIONS:

AMMONIUM AND NITRATE

1. INTRODUCTION

The present report describes scientific investigations and method evaluation for the determination of the chemical parameters: ammonium and nitrate in three different matrices: soil, biowaste and sewage sludge.

The extraction process was evaluated using 1M and 2M KCl as extraction solution for fresh and dry samples.

For method details see Annex 3 in Desk Study 16 and the following detailed description.

2. MATERIALS AND METHODS

2.1 Apparatus:

Continuous-Flow Analyzer ; manufacturer. Skalar, NL-Breda
End-over-end shaker for 100 ml glass vessels, LUFA Kassel
Ultra-sonification bath

2.2 Equipment:

100 glass vessels for extraction
filter papers free of nitrogen, manufacturer: Sartorius GmbH
product no.: 289, qualitative, for crystalline samples

2.3   Chemicals:

All Chemicals were purchased from Merck-VWR International
except KCl p.a. (Roth KG, Karlsruhe)

Extractant:.

KCl, p.a. MW: 74,56 g/mol
purity > 99,5 %

Standard addition:

KNO_3; MW = 101,11 g/mol
purity : > 99 %
NH_4Cl, MW = 53, 49 g/mol
purity. > 99,8 %

CFA analysis:

Potassiumsodiumartrate ($C_4H_4O_6KNa*4H_2O$), p.a.
(Merck Art.Nr. 1.08087)

Sodiumcitrate ($C_6H_5O_7Na*2H_2O$), p.a.
(Merck Art.Nr. 1.06448)

Sodiumhydroxide (NaOH), p.a.
(Merck Art.Nr. 1.06498)

H_3PO_4, w=85%, p.a. HCl, w=32%, p.a.

Brij-35, w=30%
(Skalar Art.Nr. 13900)

Hydrazinesulfate ($N_2H_6SO_4$), p.a.
(Merck Art.Nr. 1.04603)

Coppersulfate ($CuSO_4*5H_2O$), p.a.
(Merck Art.Nr. 1.02790)

Sulfanilamide ($C_6H_8N_2O_2S$), p.a.
(Merck Art.Nr. 1.11799)

α-Naphtylethylendiamindihydrochloride ($C_{12}H_{16}C_{12}N_2$), p.a.
Merck Art.Nr. 1.06237)

HCl, w=32%, p.a.
(z.B. Merck Art.Nr. 1.00319)

Sodiumnitroprusside ($Na_2[Fe(CN)_5NO]*2H_2O$), p.a.
Merck Art.Nr. 1.06541)

Sodiumdichlorisocyanurate ($C_3N_3O_3Cl_2Na$), p.a.
(z.B. Sigma Art.Nr. 121K3449)

Sodiumsalicylate ($C_7H_5NaO_3$), p.a.
(z.B. Merck Art.Nr. 1.06601)

Solutions for nitrate analysis:

Buffer solution: 33 g potassiumsodiumtartrate and 24 g sodiumcitrate are dissolved in a 1000 ml flask in 800 ml water, 3ml Brij is added and the flask is filled to volume with water.

Sodiumhydroxide solution: 6 g sodiumhydroxide is dissolved in a 100 ml flask in 80 ml water, 3ml Brij is added and the flask is filled to volume with water.

Coppersulfate solution: 12 g coppersulfate is dissolved in a 1000 ml flask in 800 ml water and finally filled to volume with water.

Hydrazinesulfate solution: 5 g hydrazinesulfate is dissolved in a 1000 ml flask in 800 ml water, 1,5 ml coppersulfate solution (see above) is added and the flask is filled to volume with water.

Colour reagents: 100 ml phosphoric acid is given into a 1000 ml flask filled with 700 ml water, 10g sulfanilamide and 0,5g α-naphtyl-ethylendiamindihydrochloride is added and dissolved and finally the flask is filled to volume with water.

Solutions for ammonium analysis:

Buffer solution: 33 g potassiumsodiumtartrate and 24g sodiumcitrate are given into a 1000 ml flask containing 800 ml water, 3ml Brij is added and the flask is filled to volume with water. pH is adjusted with HCl to pH 5,2 ($\pm$0,1).

Sodiumsalicylate solution: 25g sodiumhydroxide is dissolved in a 1000 ml flask in 800 ml water, 80 g Sodiumsalicylate is added and the flask is filled to volume with water.

Sodiumnitroprusside solution: 0,1g sodiumnitroprusside is dissolved in a 100 ml flask in 80 ml water and the flask is filled to volume with water.

Sodiumdichloroisocyanurate solution: 1g sodiumdichloroisocyanurate is dissolved in a 500 ml flask with water.

2.4   Samples:

Fresh samples were taken in february and march 2005 at sample sites in Hessen (Germany).

Sample No.	% residual water **	Characterization LUFA Kassel	Colour and Scent
Sandy garden soil * Eschwege	23	SO K1	light brown, sticky
Sandy soil * LUFA Speyer	1	SO K3	sandy
Composted grass and cuttings , Fulda	39	CW KF	dark brown
Composted biowaste Geismar	27	CW KG	dark brown
Sewage sludge 1	72	SL 19K	dark brown
Sewage sludge 2	77	SL 20K	dark brown,

Additional chemical parameters of samples are given on page 89
* Soil samples were taken from the top soil layer (0 –30 cm)
Samples were stored at – 8 °C prior analysis. After thawing at 4 °C over night, samples were analyzed within one day. ** residual water was determined by drying subsamples of 50 g (fresh weight) at 105 °C over night. Biowaste samples were delivered in test portions of same origin, but with differing amounts of analytes, which is stated in the tables.

Dried samples:

ISPRA sample No SO9: German soil from Hagen, ball milled < 125 μm, 0,42 % N

Standard sandy soil Kassel , originating from WEPAL ringtest 2003, < 2 mm, 0,31 % N

2.5.   Sample pretreatment:

Fresh samples were taken at temperatures below 10 °C in the field. Soil samples were taken from the upper 30 cm to 0 cm of garden - or agricultural top soil.
Transportation to the laboratory was done using a cool box at 4 °C. Samples were subdivided into aproppriate test portions at room temperature and immediately deep frozen at   -20 °C. Samples were thawed in a refrigerator prior extraction for a amximum of 18 hours.

2.6   Extraction:

A test portion of 5,0 g of sample was weighed into a 100 ml glass vessel. 50 ml of 1M KCl (or 2M KCl) were added and the glass vessel was shaken for 1 hour at room temperature using an end–over-end shaker to ensure a high extraction efficiancy. The suspension was filtrated using filter papers free of nitrogen fractions (duration of filtration was 1 hour) and finally the filtrate was filled to 100 ml with destilled water. Dried samples were processed both by filtration and centrifugation and the supernatant was used for further analysis Subsamples of each filtrate were either analyzed the same day or deep frozen at -20 °C prior analysis. Thawing of filtrates lasted less than 0,5 h at room temperature.

Preliminary experiments were performed on variations in sample amounts, amount of extractant and extraction time. Details are given in separate tables. Purity of reagents and equipment was checked by running the whole procedure without sample matrix using 1M KCl, only.

Stability tests of filtrates were performed at room temperature, 4 °C and – 18° C using a biowaste sample and synthetic stock solution of NO_3-N as KNO_3 and NH_4-N as NH_4Cl in 1M KCl.

Recovery experiments were performed on soil and biowaste/sewage sludge samples seperately using natural concentrations of of NO_3-N and NH_4-N for the addition experiment. Dried samples were used for testing the adsorption of standard addition solution by repeated extraction.

2.7   Analysis.

Analysis was performed according to EN 14256-2 using the automated continuous –flow-system.

2.8   Analytical method evaluation:

Intrinsic method parameters (range, linearity, recovery) were determined for all matrices:
soil, biowaste and sewage sludge.The concentration range of the method was chosen up to a maximum content of : NH_4-N and NO_3-N: 1200 mg/kg dry weight.
Linearity was evaluated by using 5 data points in two replicates in the range given above
by preparation and analysis of KNO_3 and NH_4Cl test solutions. Standard addition was performed by using aproppriate amounts of KNO_3 and NH_4Cl in single and two to three repeated extractions of each sample of soil, biowaste and sewage sludge and recovery was calculated.

The limit of quantification was determined using a soil sample with low contents of nitrate and ammonia (SO K3) according to DIN 32645 and ICH –guidelines.The sample was measured in ten subsamples and standard deviation was calculated. The LOQ could not be determined seperately for compost and sludge in the same way, as no sample with similar low contents of ammonium was available.

Precision measurements were performed by the analysis of six subsamples of one sample on two days.

Data analysis was done according to D.A.R. and I.C.H. guidelines (see Annex).

3.   RESULTS

3.1   Scientific investigations

Purity of reagents:

Purity of reagents and equipment was tested before evaluation and results are given below:
Nitrate-N: $< 0,05$ mg/l ; Ammonium-N.: $< 0,05$ mg/l. Data are mean values of four sample extractions in both 1M and 2M test solution

Type of extractant:

We performed extractions of all sample matrices in 1M and 2M KCl solutions in a ratio of 1 : 10. Our results indicated, that extractions using 2M KCl solution do not improve efficiency compared to extractions with 1M KCl. We therefore decided to use 1M KCl throughout the following procedures (Tables 1, 2)

Ratio of sample to extractant

A ratio of 1 : 5 (fresh or dry sample to extraction solution) resulted in significantly lower contents of extractable ammonium and nitrate in biowaste and sewage sludge samples compared to a ratio of 1 : 10. Extraction of soil samples in a ratio of 1:5 can be reasonable, if contents are near the LOQ. Here we used a ratio of 1:10 for all sample matrices, even though we were able to show, that a ratio of 1:20 in extractions of sewage sludge samples results in higher recovery values (Table 2).

Repetition of extraction

We evaluated repetition of extraction for soil and biowaste samples. Soil samples tested were dried prior analysis and extracted for 3 x 1 hour. Repetition of extraction increased recovery values for a ball milled soil sample < 125 μm, only. Dried soil samples < 2 mm could be extracted sufficiently by a single step procedure using 1M KCl in a ratio of 1:10

(Recovery: 1 step: 90%; 2. step: 92%) A fresh biowaste sample showed a slight increase in recovery, when extracted in two steps (1.step: 87%; 2. step: 92%). (Table 2)

We therefore propose to perform extractions for two sample categories:

Category A: fresh samples of different grain size and dry soil samples < 2 mm in a single step extraction and

Category B: fresh and dry biowaste and sewage sludge samples and dry soil samples < 250 μm in repeated extractions with a minimum of 2 x 1 hour of extraction.

Extraction time

We tested the influence of prolonged extraction time: 1 hour versus 2 hours and 1 hour + standing at room temperature over night. Prolonged extraction times exceeding three hours should be avoided, due to loss of analytes (Table 2). Increase of extraction time to two hours per step did not result in significantly higher extractable amounts of ammonium and nitrate.

Processing of dry samples

Both centrifugation and filtration was tested in the seperation process of dried samples.
Results obtained by filtration were significantly higher than results for centrifugation.
Still, centrifugation shows better practical performance for repeated extractions (Table 4).

Recovery

Recovery for nitrate was > 87 % in all samples tested and > 95% for extractions of dry soil
samples. Recovery for ammonium was > 90% for extractions of dry soil samples and > 80% for
extractions of fresh biowaste and sludge samples (single step extraction) (Table 2)
Adsorption tests using repeated addition of KNO_3 and NH_4Cl showed, that at each step 91 % –
98% of the added analytes could be recovered.
Recovery values for the extraction of ammonium in biowaste and sewage sludge samples were
much lower and in accordance with data published elsewhere (Laima et.al. (1999)).

Stability of filtrates

Filtrates of biowaste samples were stable for a minimum of 30 days at 4°C or – 18°C.
Filtrates should be analyzed the same day or deep frozen immediately after extraction.
(Table 3)

Precision

Relative standard deviation was highest for soil samples with low contents of ammonium and
nitrate and a maximum grain size of 10 mm: s rel = 18 %. Relative standard deviation for
biowaste and sewage sludge samples varied between 1,5 % and 13 %. (Table 5).
Dry and homogeneous samples with small grain size < 2 mm showed a maximum relative
standard deviation of 2,5 %.

LOQ

The limit of quantification in CFA analysis was 0,05 mg/l test solution for both ammonium and
nitrate. The LOQ calculated according to DIN 32645 was 0,2 mg/kg dry weight for both NH_4-N
and NO_3-N, while the LOD was 0,06 mg/kg d.w.

Linearity

The Pearsson coefficient r = 0,99946 for NH_4-N and r = 0,9975 for NO_3-N was found for a
standard curve using KNO_3 and NH_4Cl in the concentration range 0 – 214 mg/l test solution.

Table 1: Type of extraction ; Ratio of sample to extractant

Sample: Biowaste   CW KF (test portion 1)

1M KCl

Ratio sample f.w. / Extractant	Extraction time (hour)	NH_4-N (mg/kg)	NO_3-N (mg/kg)
1 : 5	1 x 1	109 +/- 2	< LOQ
1 : 10	1 x 1	139 +/- 5	< LOQ

2M KCl

Ratio sample f.w. / Extractant	Extraction time (hour)	NH_4-N (mg/kg)	NO_3-N (mg/kg)
1 : 5	1 x 1	126 +/- 2	< LOQ
1 : 10	1 x 1	135 +/-6	< LOQ

Sample: Biowaste CW KG (test portion 1)

1M KCl

Ratio sample f.w. / Extractant	Extraction time (hour)	NH_4-N (mg/kg)	NO_3-N (mg/kg)
1 : 10	1 x 1	143 +/- 7	5,6 +/- 0,1
1 : 20	1 x 1	135 +/- 7	6,8 +/- 0,1

2M KCl

Ratio sample f.w. / Extractant	Extraction time (hour)	NH_4-N (mg/kg)	NO_3-N (mg/kg)
1 : 10	1 x 1	98 +/- 7	6,1 +/- 0,1

Each data point represents the mean of 2 - 4 extractions

Recovery was estimated by standard addition of KNO_3 and NH_4Cl to fresh matrix samples

56   HORIZONTAL - 16

Continuation of Table 1

Sample: Sewage Sludge SL 19K

1M KCl

Ratio sample f.w. / Extractant	Extraction time (hour)	NH_4-N (mg/kg)	NO_3-N (mg/kg)
1 : 10	1 x 1	685 +/- 17	< LOQ
1 : 20	1 x 1	753 +/- 17	< LOQ

2M KCl

Ratio sample f.w. / Extractant	Extraction time (hour)	NH_4-N (mg/kg)	NO_3-N (mg/kg)
1 : 10	1 x 1	730 +/- 17	< LOQ

Sample: Soil SO K3

1M KCl

Ratio sample f.w. / Extractant	Extraction time (hour)	NH_4-N (mg/kg)	NO_3-N (mg/kg)
1 : 10	1 x 1	0,27 +/- 0,3	< LOQ

2M KCl

Ratio sample f.w. / Extractant	Extraction time (hour)	NH_4-N (mg/kg)	NO3_N (mg/kg)
1 : 10	1 x 1	0,22 +/- 0,3	< LOQ

Table 2: Variation of Extraction conditions – Repetition of extraction

Fresh Sample: Biowaste CW KG + addition of KNO_3 and NH_4Cl

KCl	Ratio: sample / extractant	Shaking time (hours)	NH_4-N % Recovery
2 M	1 : 5	1 x 1 h	71 +/- 6
2 M	1 : 10	1 x 1 h	85 +/- 7
2 M	1 . 5	2 x 1 h	87 +/- 7
2 M	1 . 10	2 x 1 h	92 +/- 7
1 M	1 : 5	1 x 1h	66 +/- 6
1 M	1 : 10	1 x 1h	80 +/- 7
1 M	1 : 10	2 x 1h	81 +/- 7
1 M	1 . 10	1 x 2h	76 +/- 6
1 M	1 : 10	1 x 1h + 16h ** stand at 20°C	60 +/- 6

Recovery for nitrate was > 87 % in all samples investigated

Recovery was estimated by addition of 50 mg/l NH_4-N and NO_3-N to the extraction solution

** The filtrate was left at room temperature over night to test the stability of filtrates

Each data point represents the mean of 2-6 extractions

Continuation of Table 2:

Dry sample: soil SO K3

KCl	Ratio: sample / extractant	Shaking time (hours)	NH_4-N (mg/kg)	NO_3-N (mg/kg)
1 M	1 : 10	1 x 1 h	0,20	0,32
1 M	1 : 10	2 x 1 h	0,27	0,32
1 M	1 : 10	3 x 1 h	0,27	0,32

Dry sample: soil SO K3

KCl	Ratio: sample / extractant	Shaking time (hours)	NH_4-N Recovery %	NO_3-N Recovery %
1 M	1 : 10	1 x 1 h	90	104
1 M	1 : 10	2 x 1 h	92	104
1 M	1 : 10	3 x 1 h	92	104

Dry sample: soil WEPAL standard (< 2 mm)

KCl	Ratio: sample / extractant	Shaking time (hours)	NH_4-N (mg/kg)	NO_3-N (mg/kg)
1 M	1 : 10	1 x 1 h	2,25	2,37
1 M	1 : 10	2 x 1 h	2,50	2,49
1 M	1 : 10	3 x 1 h	2,57	2,52

Dry sample: soil WEPAL standard (< 2 mm)

KCl	Ratio: sample / extractant	Shaking time (hours)	NH_4-N Recovery %	NO_3-N Recovery %
1 M	1 : 10	1 x 1 h	96	96
1 M	1 : 10	2 x 1 h	98	101
1 M	1 : 10	3 x 1 h	98	101

Each data point represents the mean of 4 extractions (s rel: < 2%)

Recovery was estimated by standard addition of 5 mg/l NO_3-N and NH_4-N to the extraction solution. S rel: 2 % - 7 %

Continuation of Table 2:

Dry sample: soil SO 9, < 125 μm

KCl	Ratio: sample / extractant	Shaking time (hours)	NH_4-N (mg/kg)	NO_3-N (mg/kg)
1 M	1 : 10	1 x 1 h	5,64	3,82
1 M	1 : 10	2 x 1 h	6,58	4,15
1 M	1 : 10	3 x 1 h	6,85	4,30

Dry sample: soil SO 9, < 125 μm

KCl	Ratio: sample / extractant	Shaking time (hours)	NH_4-N Recovery %	NO_3-N Recovery %
1 M	1 : 10	1 x 1 h	94	98
1 M	1 : 10	2 x 1 h	102	106
1 M	1 : 10	3 x 1 h	102	108

Each data point represents the mean of 2-4 extractions; S rel: < 2%

Table 2b

Adsorption efficiency for ammonium in different matrices

Sample	1. Addition	2. Addition	3. Addition
SO K3	4,62	4,72	4,92
SO9	4,55	4,64	4,89
Standard WEPAL	4,68	4,48	4,84

At each extraction step 5mg/l NH_4-N was added

Each data point represents the mean of 2-4 extractions; S rel: < 2%

Table 3: Stability of filtrates

Test mixture of KNO_3 and NH_4Cl in 1M KCl

T = 4 °C

Time (days)	NH₄-N mg/kg	NO₃-N mg/kg
0	46,6 +/- 0,5	44,8 +/- 0,5
22	46,1 +/- 0,5	44,7 +/- 0,5
30	46,7 +/- 0,5	46,9 +/- 0,5
42	44,4 +/- 0,4	44,4 +/- 0,4
56	41,2 +/- 0,4	47,4 +/- 0,5

T = - 18 °C

Time (days)	NH₄-N mg/kg	NO₃-N mg/kg
0	46,6 +/- 0,5	44,8 +/- 0,5
22	46,2 +/- 0,5	46,1 +/- 0,5
30	46,9 +/- 0,5	45,4 +/- 0,5
42	44,9 +/- 0,4	44,7 +/- 0,5
56	40,5 +/- 0,4	46,5 +/- 0,5

Remark: mg/kg refers to a hypothetical amount of 5g sample in 50 ml, though the test mixture was extracted without any sample matrix. Extraction, analysis in CFA and storage was performed as described for sample extraction and analysis.
Standard deviation was < 1 % for the analysis of both ions in artificial solution, as calculated by two repeated measurements in CFA

Continuation of Table 3:

Test portion 2 of CW KF (1 : 10) in 1M KCl

T = 4 °C

Time (days)	NH$_4$-N mg/kg	NO$_3$-N mg/kg
0	46,2 +/- 3,7	< LOQ
22	46,3 +/- 3,7	
30	45,4 +/- 3,6	
42	46,4 +/- 3,7	
56	45,6 +/- 3,6	

T = - 18°C

Time (days)	NH$_4$-N mg/kg	NO$_3$-N mg/kg
0	46,2 +/- 3,7	< LOQ
22	46,4 +/- 3,7	
30	46,0 +/- 3,7	
42	45,3 +/- 3,6	
56	43,4 +/- 3,5	

Table 4:

Variation in the separation process

Filtration

Sample	NH$_4$-N (mg/kg)	NO$_3$-N (mg/kg)
SO K3	0,29 +/- 0,02	0,17 +/- 0,02
SO9	5,92 +/- 0,13	3,78 +/- 0,02
WEPAL soil standard	2,42 +/- 0,07	2,37 +/- 0,07

Centrifugation

Sample	NH$_4$-N (mg/kg)	NO$_3$-N (mg/kg)
SO K3	0,22 +/- 0,02	0,32 +/- 0,02
SO9	5,64 +/- 0,13	3,82 +/- 0,02
WEPAL soil standard	2,22 +/- 0,04	2,37 +/- 0,07

Each data point represents the mean of four extractions

Table 5: Precision of Extraction - 1 x 1 hour in 1M KCl

Soil

SO K1

Sample No.	NH$_4$-N (mg/kg)
1	0,26
2	0,21
3	1,14 *
4	0,17
5	0,26
6	0,26

(NH$_4$-N) mean: 0,23 mg/kg
Std deviation: 0,04 mg/kg
s rel: 17% (near LOQ)
* outlier

Continuation of Table 5:

SO K3

Sample No.	NH_4-N (mg/kg)
1	0,16 *
2	0,13
3	0,11
4	0,11
5	0,11
6	0,09
7	0,09
8	0,09
9	0,13

% (NH_4-N) mean: 0,11 mg/kg
Std deviation: 0,02 mg/kg; s rel: 18% (near LOD)
* outlier

SO K3 - Nitrate

Sample No.	NO_3-N (mg/kg)
1	0,14
2	0,09
3	0,10
4	0,14
5	0,10
6	0,10
7	0,10
8	0,10
9	0,10

% (NO_3-N) mean: 0,11 mg/kg
Std deviation: 0,02 mg/kg; s rel: 18% (near LOD)

Biowaste

CW KG

Sample No.	NH_4-N (mg/kg)
1	151,4
2	152,1
3	153,2
4	155,2
5	156,6
6	156,3

% (NH_4-N) mean: 154, 1mg/kg
Std deviation: 2,2 mg/kg
s rel. 1,4 %

Continuation of Table 5:

CW KG - Nitrate

Sample No.	NO_3-N (mg/kg)
1	29,8
2	30,2
3	31,0
4	31,0
5	31,1
6	31,1

% (NO_3-N) mean: 30,7 mg/kg
Std deviation: 0,6 mg/kg
s rel: 2,0 %

CW KF

Sample No.	NH_4-N (mg/kg)
1	74,4
2	76,2
3	80,1
4	103,5
5	92,1
6	85,0

% (NH_4-N) mean: 85,3 mg/kg
Std deviation: 11,2 mg/kg
s rel: 13 %

Continuation of Table 5:

Sewage sludge

SL K19

Sample No.	NH_4-N (mg/kg)
1	671,8
2	666,4
3	685,7
4	708,5
5	697,0
6	679,6

% (NH_4-N) mean: 684,8 mg/kg
Std deviation: 15,8 mg/kg
s rel: 2,3 %

SL K20

Sample No.	NH_4-N (mg/kg)
1	674,3
2	696,7
3	702,1
4	696,4
5	717,5
6	796,1 *

% (NH_4-N) mean: 697,4 mg/kg
Std deviation: 15,5 mg/kg
s rel: 2,2 %
* outlier

4 SUMMARY

Extraction in KCl solution provides higher stability of filtrates and higher recovery for ammonium compared to other extractants, such as water or 0,02M Calciumchlorid.

Analysis of KCl extracts can be done using both manual and automated methods based on spectrophotometric detection. Here we performed analysis using the CFA automated method. Ion chromatographic detection is not possible due to interference of chloride from the extraction solution with the nitrate signale.

We tested variations in the ratio of sample to extractant, 1M versus 2M KCl extraction solutions, extraction time and storage of filtrates at two different temperatures (4°C and -18°C).

The method chosen (1M KCl, ratio: 1: 10; extraction time: 1 x 1 hour; conventional filtration, storage at -18°C prior analysis and CFA analysis) showed good practical performance during routine work, high recovery values for nitrate (> 87%) and moderate recovery values for ammonium (> 75%) in all matrices tested.

Still, great variation in ion contents: (0,2 mg/kg in soil and up to 700 mg/kg in sewage sludge) and contents of dry matter impose difficulties to both the extraction and analytical part.
Calibration and handling during laboratory work should be performed separately for soil and for biowaste/sewage sludge in order to prevent cross contaminations
Repeated extractions improve recovery values, especially for samples with high contents of ammonium/nitrate such as fresh biowaste and sewage sludges and for dried and ball milled soil samples used in validation.
Here, an alternative method using two repeated extractions and filtration or centrifugation is a method of choice for these sample catagories.

CHAPTER 4

AQUA REGIA EXTRACTION METHODS FOR THE DETERMINATION

OF TOTAL PHOSPHORUS

IN SOIL, SEWAGE SLUDGE AND BIOWASTE

1   INTRODUCTION

The present report describes method evaluation on extraction procedures by microwave and reflux heating for the determination of the chemical parameter: total phosphorus in three different matrices: soil, biowaste/compost and sewage sludge.

Analysis was performed using ICP-OES and ICP-MS

For method details see Annex 1 in Desk Study 16

2   MATERIALS

2.1   Apparatus:

Extraction:

Microwave: MLS 1200 , closed teflon vial size: 80 ml (3,2 cm x 10 cm)

Reflux: 250 ml glass Erlenmeyer flask connected to a reflux condenser

Analysis:

ICP-OES, Optima 3300 DV, manufacturer: Perkin Elmer instruments, D-Rottgau

ICP-MS, Perkin Elmer instruments, D-Rottgau

2.2   Chemicals:

All chemicals for routine use were obtained from Merck – VWR international

HCl p.a. 37%,
HNO$_3$ p.a. 65 %
H$_2$SO$_4$ p.a. 98%

Standards:

ICP Standard, 100 mg/l Phosphorus; additional values: Ca, Fe, K, Mg, Al, Na, Zn, Mn, Sr, Cr, Cu, Ni, Pb, Cd, Co

KH$_2$PO$_4$ in standard addition
MW = 136,09 g/mol ; purity : > 99,5 %

NIST standard; CRM 2781 (domestic sludge; 2,42 % P (+/- 0,09 % P)
RTC standard, CRM 00-050 (sludge amended soil, terra rossa; 1,01 % P (+/- 0,09 % P)
Distribution: Promochem GmbH, Wesel

Standards prepared in Kassel during ringtest validation 2002, WEPAL
Biowaste; 0,45 % P (+/- 0,05 % P); Sludge; 3,3 % P (+/- 0,1 % P)

2.3   Samples:

<u>« Playground samples » from JRC, Ispra</u>

Sample No.	Description	% P in SWCT report	Colour and Scent
SO 4 ***	Clay soil, < 125 μm	0,13	light brown
SO 13	Terra Rossa < 2 mm	0,95	light red-brown
SO 16R	Eurosoil 3R < 2 mm	0,17	light-grey-brown
CW 1	Composted garbage, < 125 μm	0,49	dark brown,
CW 5	Compost < 125 μm	0,63	dark brown,
SL 4	Sewage sludge, < 125 μm	1,99	brown, strong scent low density
SL 11***	Sewage sludge from electric Industry, < 125 μm	0,40	light blue/green low density

Samples from ISPRA were air dried and ball milled, and % P was determined by XRF analysis

*** Analysis was performed in Kassel using ICP-MS, due to low contents and interferences in ICP-OES
all other samples were analyzed by ICP-OES

% RW.: residual water determined by heating at 105°C over night:
RW (soils) < 2 %; RW biowaste < 5 % ; RW sludge: SL 4 < 5%; SL11: 12 %

2.4   Microwave assisted digestion:

2.4.1 Samples prepared in Kassel (Hessisches Landeslabor, Desk study 16) closed system

Samples were weighed into teflon vials: 400 mg (+/- 1 mg) soil and 200 mg (+/- 1 mg) sludge and biowaste and 3 ml HCl p.a.and 1 ml HNO_3 p.a. were added. Vials were closed and kept at room temperature over night (max.: 20 h). Heating was performed in a closed system using the following temperature programme: 4 min – 250 W; 2 min – 0 W; 1,3 min – 250 W; 2 min – 450 W, 5 min – 850 W; 1 min – 250 W and finally the reaction mixtures were allowed to cool and settle down for 60 min at room temperature.

The reaction mixtures were transferred to a 25 ml flask, teflon vials were rinsed with destilled water twice and the flask filled to 25 ml. Aliquots were transferred to PP vessels and allowed to settle down at room temperature. The supernatants were used for analysis

2.4.2   Samples prepared in Maria Enzersdorf (NUA Umweltanalytik, M.Kisser, desk study 18) closed and open systems

Samples were weighed into teflon vials: 500 mg (+/- 1 mg) soil and 500 mg (+/- 1 mg) sludge and biowaste and 6 ml HCl (suprapur) and 2 ml HNO_3 (suprapur) were added.

Heating was performed using the following temperature programme:

2 min – 250 W; 2 min – 0 W; 5 min – 250 W; 5 min – 450 W, 5 min – 850 W;

and finally the reaction mixtures were allowed to cool and settle down for 60 min at room temperature.

The reaction mixtures were transferred to a 50 ml flask, teflon vials were rinsed with double-destilled water twice and the flask filled to 50 ml. Aliquots were transferred to PP vessels and allowed to settle down at room temperature. The supernatants were used for analysis

2.5 Reflux digestion

2.5.1 Samples prepared in Kassel (Desk study 16)

Samples were weighed into a 250 ml Erlenmeyer flask : 3,00 g (+/- 0,01g) soil and 1,50 g (+/- 0,01g) sludge and biowaste and 21 ml HCl p.a.and 7 ml HNO_3 p.a. were added. The amount of biowaste and sludge used prevented the digestion mixture from excessive foaming. Erlenmeyer were connected to the condenser system and kept at room temperature over night (max.: 20 h). Heating at reflux was performed for 2 hours and finally the reaction mixtures were allowed to cool and settle down for 60 min. The reaction mixtures were transferred to a 100 ml flask and the flask filled to 100 ml with destilled water. Aliquots were transferred to PP vessels and allowed to settle down at room temperature. The supernatants were used for analysis.

Digestions with sulfuric acid and nitric acid were performed according to prEN14672

2.5.2 Samples prepared in Maria Enzersdorf (desk study 18)

Samples were weighed into a 250 ml Erlenmeyer flask: 3,00 g (+/- 0,01 g) soil and 3,00 g (+/- 0,01 g) sludge and biowaste , the samples were moistened with 0,5 ml destilled water and 21 ml HCl (suprapur) and 7 ml HNO_3 (suprapur) were added. Erlenmeyer were connected to the condenser system and kept at room temperature over night (max.: 20 h). Heating at reflux was performed for 2 hours and finally the reaction mixtures were allowed to cool and settle down for 60 min at room temperature. The reaction mixtures were transferred to a 200 ml flask and the flask filled to 200 ml with destilled water. Aliquots were transferred to PP vessels and allowed to settle down at room temperature. The supernatants were used for analysis

2.6   Scientific investigations:

Microwave assisted digestion methods were evaluated and their comparability was studied.
Samples digested in Maria Enzersdorf at NUA Umweltanalytik (M. Kisser) and in Kassel
at Hessisches Landeslabor (E. Janssen) were exchanged and analyzed for contents of total
phosphorus by ICP in Kassel.

The efficiency and precision of digestions in both closed and open microwave systems from
different manufacturers were tested. Digestion time and temperature programme used varied
between samples digested in Kassel and Maria Enzersdorf. Furthermore, variations in the ratio
of sample to extractant were evaluated using ratios of 1 : 10 ; 1 : 15 ;1 : 20 and 1 : 40. Results
obatined from microwave assisted digestion and conventional reflux digestion were compared.
Scientific investigations on reflux digestion were carried out on the comparability of results
from digestions using H_2SO_4/HNO_3 according to prEN 14672 and Aqua regia.

2.7   Analytical evaluation:

For both procedures – microwave and reflux digestion – recovery and precision data were
evaluated.Recovery was estimated using NIST certified reference materials of sludge and sludge
amended soil (Terra Rossa). Precision was measured by the analysis of three digested
subsamples of each sample provided by ISPRA and by 6 – 10 digested subsamples of each
reference standards.

Additionally, recovery was calculated from standard addition experiments using a sludge
standard prepared during WEPAL Enquete 2002 containing 3,32 % P and addition of synthetic
KH_2PO_4 (with a purity of 99,5% and a posphorus content of P: 22,8%)

Linearity and range were calculated from a standard curve prepared by microwave digestion of
KH_2PO_4 up to a maximum content of 9% P in sludge and biowaste and 4,5 % P in soil samples.

Linearity and range of the conventional reflux method was determined by standard addition up
to a maximum content of 4,8 % P in sludge and biowaste and 1,0 % P in soil.

Linearity of the analytical ICP method was determined by preparation of a standard curve of
a synthetic multi-element standard in aqua regia matrix solution.

The LOQ was calculated from standard deviations according to DIN 32645 using sample
No SO4 and SO16R with low contents of total P.

3. RESULTS:

3.1 Variation in the ratio: sample to extractant

Ratios of sample to extractant (1: 10 to 1 : 40) have been evaluated by microwave assisted heating (Table 1). Results for soil samples did not differ between treatments, while biowaste and sludge digestions showed higher efficiency in treatments with ratios above 1 : 10. We therefore chose a ratio of 1 : 10 for soil samples and 1: 20 for biowaste and sludge throughout both reflux and microwave assisted heating. All samples digested at NUA Umweltanalytik (M. Kisser) were digested with a ratio of 1 : 10 (reflux) and 1 : 16 (microwave).

Results for samples with a phosphorus content < 0,5 % did not differ between reflux digestions performed using a ratio of 1 : 10 or 1 : 20.

3.2 Comparability of results obtained from different microwave systems

Microwave systems were tested in Kassel and Maria Enzersdorf (Tabel 2 and 3). Results for total phosphorus in soil samples did not vary significantly between both laboratories using closed and open microwave systems from two manufacturers. Total phosphorus contents for biowaste CW1 were significantly increased in the digestion performed in Kassel compared to M. Enzersdorf, while results for soil sample SO13 (Terra Rossa, < 2mm) were lower in Kassel compared to M. Enzersdorf, though differences for soil samples were not statistically significant. Both closed and open microwave systems were tested in M. Enzersdorf. Mean values did not differ between treatments.

3.3 Reflux digestion versus microwave digestion

Results obtained from both microwave and reflux heating showed good recovery (> 96%) - estimated by digestion of standard reference soil and sludge samples with total phosphorus contents > 1%.

Mean values among both methods: reflux versus microwave did not differ significantly for the digestion of samples from ISPRA.

Still, we do not recommend microwave assisted digestions during routine work, due to the corrosive action of aqua regia on mechanical parts of the digestion system.

3.4 Sulfuric acid/nitric acid versus aqua regia digestion (reflux)

Results for the digestion of soil and biowaste/ sewage sludge samples with sulfuric acid / nitric acid did not differ significantly from results obtained by aqua regia digestion, though mean values were slightly increased and visible inspection of residues after digestion showed better efficiency after digestion in sulfuric acid/nitric acid (Table 6, 8).

3.5 Linearity

Linearity of the chemical standard KH_2PO_4 in microwave digestion was analyzed in the concentration range 0 – 9% P in sewage sludge and biowaste as calculated for 200 mg used in real matrix samples and for 400 mg in soil samples Linearity was proven by a Pearsson coefficient of r = 0,997. (x-axis: mg P, determined by weight; y-axis: mg/l P found after digestion.)

3.6 Limit of quantification

The LOQ in ICP-OES was 0,10 % P, while the LOD was 0,04 % P.
Calculation was based on the standard deviation of soil sample SO16R and intrinisic method parameters of ICP-OES analysis (LOQ: < 20 mg/l solution (1+9 dilution).)

3.7 Recovery

Recovery in soil varied between 100,0 % and 112 %.
Recovery in biowaste and sludge varied between: 93,0 % and 98,8 %.
(see Tables)

3.8   Precision

Soil, compost and sewage sludge samples varied in phosphorus contents between 0,05% - 0,2%P (SO4, SO16R), 0,3 % – 0,6% P (CW1, CW5, SO13, SL11) and > 1 %P (SL4) and showed a maximum relative standard deviation of 17 % (see Tables 1 – 4).

Relative standard deviation of sample digestion using reflux heating generally was lower than digestion using microwave assisted heating - 2 – 10% (reflux) versus 3 – 14% (microwave).

(Table 7)

Table 1: Variation of sample to extractant in microwave digestion

SO16R

Ratio	% P
1 : 10	0,14
1 : 15	0,13
1 : 20	0,14
1 : 40	0,13

Stddev.:+/- 0,01 %P

CW1

Ratio	% P
1 : 10	0,33
1 : 15	0,40
1 : 20	0,44
1 : 40	0,46

Stddev.: +/- 0,08 %P

SL4

Ratio	% P
1 : 10	2,15
1 : 15	2,15
1 : 20	2,39
1 : 40	2,33

Stddev. : +/- 0,20 %P

Results are mean values of 2 – 4 digestions

Table 2:

Precision of samples digested in Kassel by closed microwave - MLS system

Soil

Sample No.	% P
SO4	0,09 +/- 0,01 **
SO13	0,69 +/- 0,08
SO16R	0,14 +/- 0,02

** Analysis was performed by ICP-MS
Each data point represents the mean of three digestions
s rel: 11 % – 14 %

Biowaste

Sample No.	% P
CW1	0,44 +/- 0,03
CW5	0,53 +/- 0,01

Each data point represents the mean of three digestions
s rel: 2 % - 5 %

Sludge

Sample No.	% P
SL4	2,40 +/- 0,25
SL11	0,56 +/- 0,05

Each data point represents the mean of three digestions
s rel: 9 % - 11 %

Table 3: Recovery in certified reference materials – microwave digestion

Sludge amended soil CRM 005-050

Sample No.	% P
1	1,16
2	1,06
3	1,21
4	1,20
5	1,10
6	1,13
7	1,08
8	1,13
9	1,10
10	1,10

% P mean: 1,13   % P
Std deviation : 0,05 % P   informative value: 1,01 +/- not. det. %P
s rel: 6 %

Domestic sewage sludge – NIST 2781

Sample No.	% P
1	2,38
2	2,38
3	2,40
4	2,43
5	2,42

% P mean: 2,40 % P   Recovery: 99 %
Std deviation: 0,05 % P   certified value: 2,42 +/- 0,09   %P
s rel: 12 %

Table 4:
Precision in additional samples from NUA Umweltanalytik

Digestion in both closed and open microwave systems

SO4 – open system

Sample No.	% P
1	0,08
2	0,09
3	0,09
4	0,08
5	0,09

% P mean: 0,09 % P
Std deviation : 0,01 % P
s rel: 11 %

SO4 – closed system

Sample No.	% P
1	0,09
2	0,09
3	0,09
4	0,08
5	0,09

% P mean: 0,09 % P
Std deviation: 0,01 % P
s rel: 11 %

SO16R – open system

Sample No.	% P
1	0,15
2	0,16
3	0,16
4	0,14
5	0,15

% P mean: 0,15 % P
Std deviation : 0,01 % P
s rel: 7 %

SO16R – closed system

Sample No.	% P
1	0,15
2	0,16
3	0,15
4	0,15
5	0,15

% P mean: 0,15 % P
Std deviation: 0,01 % P
s rel: 7 %

SO13 – open system

Sample No.	% P
1	1,09
2	0,86
3	0,72
4	0,89
5	0,79

% P mean: 0,87 % P
Std deviation: 0,15 % P
s rel: 17 %

SO16R – open system

Sample No.	% P
1	0,15
2	0,16
3	0,16
4	0,14
5	0,15

% P mean: 0,15 % P
Std deviation : 0,01 % P
s rel: 7 %

SO16R – closed system

Sample No.	% P
1	0,15
2	0,16
3	0,15
4	0,15
5	0,15

% P mean: 0,15 % P
Std deviation: 0,01 % P
s rel: 7 %

CW1 – open system

Sample No.	% P
1	0,42
2	0,45
3	0,44
4	0,45
5	0,45

% P mean: 0,45 % P
Std deviation : 0,02 % P
s rel : 4 %

CW1 – closed system

Sample No.	% P
1	0,44
2	0,44
3	0,44
4	0,44
5	0,44

% P mean: 0,44 % P
Std deviation: 0,01 % P
s rel: 2 %

Table 5: Precision - Reflux digestion using aqua regia

Soil

Sample No.	% P
SO4	0,06 +/- 0,01 **
SO13	0,78 +/- 0,08
SO16R	0,11 +/- 0,01

** Analysis was performed by ICP-MS

Each data point represents the mean of three digestions
s rel: 9 % - 17 %

Biowaste

Sample No.	% P
CW1	0,41 +/- 0,01
CW5	0,52 +/- 0,03

Each data point represents the mean of three digestions
s rel: 2 % - 6 %

Sludge

Sample No.	% P
SL4	2,55 +/- 0,25
SL11	0,68 +/- 0,05

Each data point represents the mean of three digestions
s rel: 7 % - 10 %

Table 6: Recovery in certified reference materials – Reflux digestion

Sludge amended soil CRM 005-050

Sample No.	% P
1	1,08
2	1,09
3	1,05
4	1,06
5	1,05
6	1,03
7	1,06
8	1,07
9	1,07
10	1,03

% P mean: 1,06 % P
Std deviation : 0,02 % P   informative value: 1,01 +/- std.dev.not.determined
s rel: 2 %

Domestic sewage sludge – NIST 2781

Sample No.	% P
1	2,31
2	2,29
3	2,28
4	2,30
5	2,37

% P mean: 2,31 % P   Recovery: 96 %
Std deviation: 0,03 % P   certified value: 2,42 +/- 0,09 %P
s rel: 2 %

Table 7: Precision – Reflux digestion using aqua regia
Analysis of additional samples from NUA Umweltanalytik

SO4

Sample No.	% P
1	0,06
2	0,06
3	0,06
4	0,06
5	0,06

% P mean: 0,06 % P
Std deviation : 0,01 % P
s rel: 17 %

SO16R

Sample No.	% P
1	0,10
2	0,10
3	0,10
4	0,10
5	0,10

% P mean: 0,10 % P
Std deviation : 0,01 % P
s rel: 10 %

SO13

Sample No.	% P
1	0,74
2	0,76
3	0,73
4	0,76
5	0,76

% P mean: 0,75 % P
Std deviation: 0,02 % P
s rel: 3 %

CW1

Sample No.	% P
1	0,37
2	0,39
3	0,39
4	0,39
5	0,38

% P mean: 0,38 % P
Std deviation: 0,01 % P
s rel: 3 %

Table 8: Reflux digestion with sulfuric acid / nitric acid

SO13

Sample No.	% P
1	0,79
2	0,80
3	0,91

% P mean: 0,83 % P
Std deviation: 0,07 % P
s rel: 8 %

CW1

Sample No.	% P
1	0,45
2	0,47
3	0,50

% P mean: 0,47 % P
Std deviation: 0,02 % P
s rel: 4 %

SL11

Sample No.	% P
1	0,80
2	0.80
3	0,66

% P mean: 0,75 % P
Std deviation: 0,06 % P
s rel: 8 %

4   SUMMARY

We evaluated two different reflux digestion methods (aqua regia versus sulfuric acid/nitric acid) and two microwave digestion methods (aqua regia assisted by microwave heating in open and closed systems). No significant difference in contents of total phosphorus could be found for reflux versus microwave digestion or closed versus open microwave digestion.

Furthermore reflux digestion using sulfuric acid/nitric acid showed slightly increased but not statistically significant differences compared to reflux digestion in aqua regia.

We preferred conventional reflux digestion, as microwave assisted digestion using aqua regia causes corrosive damage to mechanical parts. Still, microwave heating may be a good choice for small sample amounts in small experimental series.

Variations in the ratio of sample amount to aqua regia was performed and as a result we propose a ratio of 1: 10 for soil samples and a ratio of at least 1 : 20 for biowaste and sludge samples for the analysis of phosphorus. In multielement analysis (phosphorus + trace elements) a ratio of 1 : 10 in reflux digestion is preferred, as concentrations of certain trace elements are near the LOQ.

Recovery was > 93% for all matrices tested and precision data showed a maximum relative standard deviation of 18 % for soil samples with low contents of total P and a maximum of 10 % for biowaste and sewage sludge samples.

Description of additional samples from Kassel

Soil SO K1: Soil type: loamy sand; < 10 mm; Origin: Germany, Eschwege; used fresh

Soil SO K3: Soil type: Sand; < 2 mm; Origin: Germany, Speyer; used air dried

Standard soil SO WEPAL: Soil type: sandy Loam; < 2 mm; Origin: WEPAL; used air dried

Documentation: LUFA Speyer and LUFA Kassel (Seibert, Ellinghaus, pers. comm., 2005)

Biowaste Kas1: Garden Compost; < 2 mm; Origin: Germany; used air dried

Biowaste Kas2: biogas fermented sludge; < 2 mm; Origin: Germany; used air dried

Biowaste Kas 3 and CW KG: Garden Compost; < 2 mm; Origin: Germany; used air dried

Biowaste: CW KF: Garden Compost; < 10 mm; Origin: Germany; used fresh

Documentation: LUFA Kassel (Janssen, 2004)

Sludge SL 19: Domestic sludge; < 2 mm; Origin: Germany; used fresh

Sludge SL 20: Domestic sludge; < 2 mm; Origin: Germany; used fresh

Documantation: LUFA Kassel (Schaaf, 2005, pers. comm.)

CALCULATIONS:

mean concentration: $\hat{y} = \dfrac{\sum y_i}{n}$

variance: $s^2 = \dfrac{\sum y_i^2 - (\sum y_i)^2 / n}{(n-1)}$

standard deviation: $s = \sqrt{s^2}$

Nalimov test $\quad r^* = \dfrac{\left| y^* - \hat{y} \right|}{s} \times \sqrt{(n / n\text{-}1)}$; FG = n – 2 ; r* >/= r (Table), then outlier

Grubbs test $\quad r^* = \dfrac{\left| y^* - \hat{y} \right|}{s}$ see also Nalimov test (according to Loszan, 1998)

Students t- test $\quad t = \dfrac{\left| x_1 - x_2 \right|}{\sqrt{\dfrac{s_1^2 + s_2^2}{n_1 + n_2}}}$ FG = $n_1 + n_2$ -2 t_{Tab} (95%, 2 sided)

$t_{calc.} < t_{tab}$, H0, no significant difference

F test: $\quad F = s_1^2 / s_2^2$ FG = n-1; Ho: $s_1^2 = s_2^2$

LOD = 3,3 x s (according to I.C.H. guidelines, 1994-1996)

LOQ = 10 x s

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DRAFT STANDARD (CEN TEMPLATE)

See Annex 1 - 4